IMMUNITY TO FUNGAL INFECTIONS

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The topic of immunity to fungal infections is of interest to a wide range of disciplines, from microbiology to immunology. It is of particular interest in terms of therapy of HIV-infected individuals, and patients with cancer or individuals who have received transplants. Understanding the nature and function of the immune response to fungi is an exciting challenge that might set the stage for new approaches to the treatment of fungal diseases, from immunotherapy to vaccines. The past decade has witnessed the development of a wide range of new approaches to elucidate events that occur at the host–fungus interface.

COMMENSALS
Two organisms that live in a close relationship, in which one benefits by the relationship and the other neither benefits nor is harmed.

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The kingdom of Fungi comprises many species that are associated with a wide spectrum of diseases in humans[1] (BOX 1). The clinical relevance of fungal diseases has increased enormously in the second half of the twentieth century, mainly because of an increasing population of immunocompromised hosts, including individuals infected with HIV, transplant recipients and patients with cancer[2–4]. The fungal threat will continue to increase, as shown by the occurrence of aspergillosis in severe acute respiratory syndrome (SARS)[5] and by the inclusion of *Coccidiodes immitis* as a potential agent of bioterrorism[6]. The crude mortality from opportunistic fungal infections still exceeds 50% in most human studies, and has been reported to be as high as 95% in bone-marrow transplant recipients infected with *Aspergillus* species. Therefore, the study of fungi is a research priority. Because fungal pathogens are eukaryotes, and therefore share many of their biological processes with humans, many antifungal drugs can cause toxicity when used therapeutically[7]. No standardized vaccines exist for preventing any of the human infections caused by fungi — a situation that is attributable to both the complexity of the pathogens and their sophisticated strategies for surviving in the host and evading immune responses (BOX 2). Although not unique among infectious agents, fungi have complex and unusual relationships with the vertebrate immune system, partly due to some prominent features. Among these are their ability to exist in different forms and to reversibly switch from one to the other during infection (BOX 2). For COMMENSALS, two prominent features are also important: the highly effective strategies of immune evasion they must have evolved to survive in the host environment and the prolonged antigenic stimulation of the host that can have marked immunoregulatory consequences. So, in the context of the antagonistic relationships that characterize host–pathogen interactions, the strategies used by the host to limit fungal infectivity are necessarily distinct and, in retaliation, fungi have developed their own elaborate tactics to sidestep these defences[1]. This highlights the complexity of the relative contributions of individual aspects of host defences in limiting fungal infectivity. However, as immune restoration after combination antiretroviral chemotherapy has been shown to limit fungal infections in HIV-positive individuals[4], manipulation of the immune system could be a candidate for future strategies aimed at preventing or treating fungal infections in susceptible patients. In this review, I outline cellular and molecular pathways of immune defence mechanisms that have greatly contributed to our present understanding of the host response to fungi from a regulatory perspective, and have been most helpful in accommodating the clinical findings in a conceptual framework that is amenable to strategies of immunointervention.

Immunity to fungi

It is known that host defence mechanisms influence the manifestation and severity of fungal infections, such that the clinical forms of the disease depend on a patient's immune response. For example, the host

Box 1 | **The pathogenesis of typical fungal infections**

Most fungal infections are accidental and originate from an exogenous source by inhalation (*Aspergillus* spp., *Cryptococcus neoformans* or endemic mycoses), the gastrointestinal tract for commensals (*Candida* spp.) or reactivation of a latent infection.

Aspergillus fumigatus
- Alveolar macrophages ingest and destroy inhaled conidia and prevent germination to branching and septate hyphae, which is the invasive form that is associated with fatal infection. Fungal proteases might enable conidia to evade phagocytosis and killing.
- As a second line of defence, neutrophils attack hyphae through the release of oxidants and degranulation.
- Pulmonary dendritic cells (DCs) ingest conidia and hyphae, migrate to draining lymph nodes and instruct local disparate T helper (T_H)-cell responses.

Cryptococcus neoformans
- The monomorphic yeast typically causes primary pulmonary infections, from where haematogenous spread to the meninges might occur together with defective T_H1-cell-mediated immunity.
- The most distinctive feature of this fungus is the presence of an acidic mucopolysaccharide capsule, which is required for virulence and is important diagnostically.

Endemic mycoses
- Primary pulmonary infection occurs as a result of inhalation of conidia, which then convert to the pathogenic yeast form or to spherules (in the case of *Coccidiodes immitis*).
- Yeasts survive and replicate in host macrophages through several evasion strategies that include defective phagosome–lysosome fusion, regulation of phagosomal pH, iron restriction, suppression of the respiratory burst and dysregulated production of pro-inflammatory cytokines.
- Patients with defective T_H1-cell-mediated immunity might suffer life-threatening progression or reactivation of latent foci of infection.

Candida albicans
- The clinical spectrum of *C. albicans* infections ranges from mucocutaneous to systemic life-threatening infections. The main risk factors that predispose to severe candidal infections are congenital or acquired defects of cell-mediated immunity, including quantitative and qualitative defects in neutrophils and dysregulated T_H-cell reactivity.
- In histopathological sections, budding yeast cells, PSEUDOHYPHAE and HYPHAE can be seen. In mice, both yeasts and hyphae translocate across the gastrointestinal tract and might eventually gain access to blood vessels.
- Gut DCs phagocytose both fungal morphotypes and instruct local and systemic T_H-cell responses. In the presence of protective opsonizing IgM, gut DCs produce interleukin-10 and activate regulatory T (T_{Reg}) cells that negatively regulate antifungal T_H1-cell reactivity. By providing signals to different T-cell subsets, including T_H and T_{Reg} cells, DCs coordinate the overall immune response at the sites of colonization/infection.

PSEUDOHYPHAE
An exaggerated form of budding in which the newly formed cells do not take on an oval shape and pinch off from the parent, but instead remain attached and continue to elongate.

HYPHAE
In moulds, spores germinate to produce branching filaments known as hyphae (singular hypha), ~2 to 10 μm in diameter, which might form a mass of intertwining strands known as mycelia.

immune system is a major determinant of which particular form of disease will develop after exposure to the ubiquitous organism *Aspergillus fumigatus*[8] or whether transition from commensalism to infection will occur with *Candida albicans*[9,10]. The host defence mechanisms against fungi are numerous, and range from protective mechanisms that were present early in the evolution of multicellular organisms ('innate immunity') to sophisticated adaptive mechanisms, which are specifically induced during infection and disease ('adaptive immunity'). The T HELPER 1 (TH1)/TH2 dichotomy has shed light on the general principle that diverse effector functions are required for eradication of different fungal infections[11–14].

Innate immunity

Traditionally considered only as a first line of defence, innate immunity has recently received renewed attention because, despite a certain lack of specificity, it effectively distinguishes self from non-self and activates adaptive immune mechanisms by the provision of specific signals[15]. The constitutive mechanisms of innate defence are present at sites of continuous interaction with fungi and include the barrier function of body surfaces and the mucosal epithelial surfaces of the respiratory, gastrointestinal and genito-urinary tracts (reviewed in REF. 16). Microbial antagonism (lactobacilli and bifidobacteria have shown efficacy in the biotherapy of candidiasis), DEFENSINS and COLLECTINS indicate the marked pathogen specificity of the constitutive mechanisms[17–19]. Most host defence mechanisms, however, are inducible after infection and, therefore, their activation requires that invariant molecular structures shared by large groups of pathogens (also known as pathogen-associated molecular patterns, PAMPs) are recognized by a set of pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), as discussed later.

Mammalian innate antifungal defences are mediated by cells, cellular receptors and several humoral factors. Professional phagocytes, consisting of neutrophils, mononuclear leukocytes (monocytes and macrophages) and dendritic cells (DCs) have an essential role. Natural killer (NK) cells, γδ T CELLS and non-haematopoietic cells, such as epithelial and endothelial cells, are also important[16]. However, their relative contributions largely depend on the site of infection. The innate response to fungi serves two main purposes: a direct antifungal effector activity by carrying out pathogen destruction through either a phagocytic process, which provides an immediate innate cellular immune response against fungi residing intracellularly, or through the secretion of microbicidal compounds against uningestible fungal elements; and an instructive role on cells of the adaptive immune system, through the production of pro-inflammatory mediators, including chemokines and cytokines, the induction of co-stimulatory activity by phagocytic cells, and antigen uptake and presentation. Although a division of labour exists among the cellular mediators of the innate system, the perception is that they might share the ability to serve both functions of the innate immune response. This might allow for full use of redundancy and compensatory mechanisms under specific conditions of infection and disease.

The TLR–PAMP recognition system. Mammalian TLRs are a family of conserved cellular receptors that mediate cellular responses to PAMPs and other ligands[20]. *Toll* was originally defined as a *Drosophila* gene that was important for ontogenesis and antimicrobial resistance[21]. The sequence similarity of the cytoplasmic portion of *Drosophila* Toll and the intracellular domains of mammalian interleukin-1 receptor (IL-1R) indicated similarities in Toll and IL-1R signalling and illustrated the evolutionary conservation of both cell-signalling systems[22].

All TLRs activate a core set of stereotyped responses, including inflammation. However, individual TLRs can also induce specific programmes — in a myeloid differentiation primary response gene 88 (MYD88)-dependent[22] or -independent manner[23] — in cells of the innate immune system that are tailored for a particular pathogen. It is recognized that the intricacies of how TLRs signal will ultimately provide an explanation for the molecular basis of how cells involved in innate immunity dictate the processes of host defence that are specific to the provoking pathogen[22].

Several cell-wall components of fungi might act as PAMPs that are recognized by TLRs expressed by phagocytes and DCs (FIG. 1). TLR2 signalling leads to the prevalent production of inflammatory cytokines, such as tumour-necrosis factor (TNF) and IL-1β, although IL-10 is also produced occasionally[24–28]. Signalling through TLR2 by zymosan occurs together with the β-glucan receptor dectin-1 (REF. 26), which indicates collaborative recognition of distinct microbial components by different classes of innate immune receptors. Although not formally proven, dectin-1 also seems to mediate the recognition of *Pneumocystis carinii* β-glucan, which is

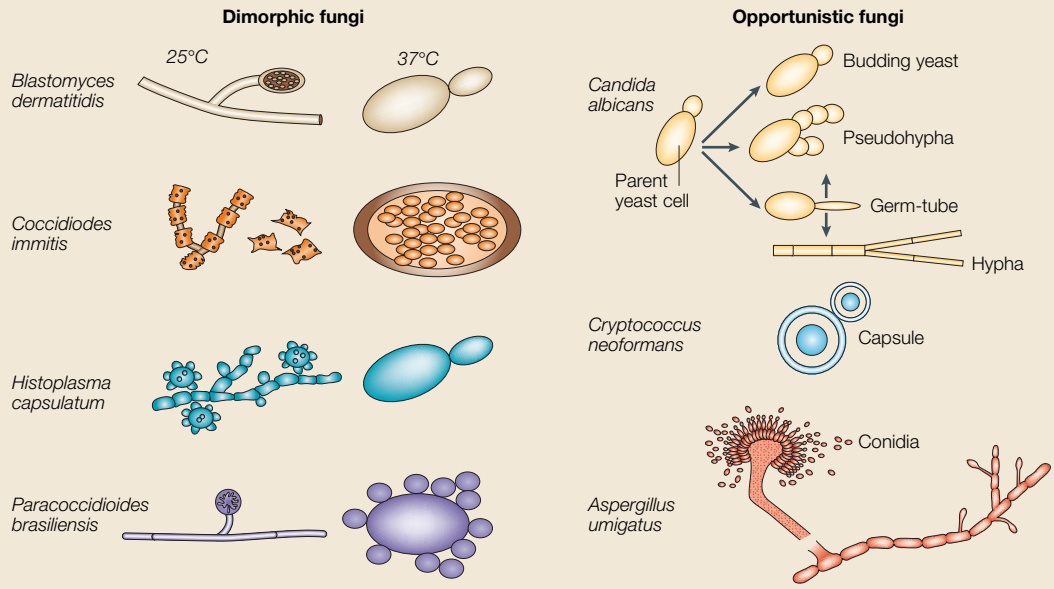
T HELPER 1 (TH1)/TH2 CELLS
Functional subsets of CD4+ T cells that express αβ-T-cell receptors. They produce either type-1 cytokines (such as IL-2 and IFN-γ) that support macrophage activation, the generation of cytotoxic T cells and the production of opsonizing antibodies or type-2 cytokines (such as IL-4, IL-5 and IL-13) that support B-cell activation, the production of non-opsonizing antibodies, allergic reactions and the expulsion of extracellular parasites.

DEFENSINS
Small basic peptides produced by immune cells that are microbicidal and work by damaging bacterial membranes.

COLLECTINS
A family of proteins so named because they contain both collagen-like sequences and calcium-dependent lectin domains. The collectins bind to various carbohydrates present on the surfaces of microorganisms through their lectin domains and also interact with cell-surface receptors on phagocytic cells to promote the uptake of bound particles.

γδ T CELLS
T cells express a T-cell receptor (TCR) composed of either α- and β-subunits (αβ-TCR) or of γ- and δ-subunits (γδ-TCR). Most (>90%) T cells have an αβ-TCR that recognizes conventional MHC class I or II molecules. T cells that express γδ-TCRs are less frequent and the ligands of this type of receptor are less well characterized.

Box 2 | Fungal infections: morphogenesis and virulence



Humans are constantly exposed to fungi, but only a limited number of fungi cause severe infections. So, pathogenicity is not a stable characteristic of most fungi. The pathogenesis of fungal infections involves several virulence factors that allow fungal survival and persistence in the host, eventually leading to tissue damage. Some virulence factors are of obvious importance, such as: various complementary structures through which fungi adhere to host tissues and the extracellular matrix; the production of phospholipases, proteases and elastases that cause tissue damage and impairment of host defences; the ability to switch to metabolic pathways that are required for intracellular survival; thermotolerance (the ability to grow at 37°C), which is a prerequisite for dissemination to visceral organs; and the ability to exist in different forms and to reversibly switch from one to the other during infection[122–124].

Examples of the latter are the dimorphic fungi, which transform from saprobic filamentous moulds to unicellular yeasts in the host. Also, some species of *Candida* can grow in different forms, such as yeasts, blastospores, pseudohyphae and hyphae, depending on infection sites. *Cryptococcus neoformans* yeasts become coated with a capsule, and the filamentous fungi (for example, *Aspergillus* spp., *Fusarium* spp. and *Zygomycota*), which are inhaled as unicellular conidia, can transform into branching hyphae in the lungs. Although such morphogenesis is an example of a developmental change, it is distinct from the life cycles that are displayed by other organisms, as there is no evidence that cycling between different morphotypes is obligatory for fungi. Instead, morphological transition, often connected with metabolic flexibility, is a mechanism that some fungi have evolved to adapt to different environments.

Associations between morphogenesis and virulence have long been presumed for dimorphic fungi that are human pathogens, as one morphotype exists in the environment or during commensalism, and others in the host during the disease process[125]. However, although morphological flexibility could be a key contributor to fungal invasion, no molecular data unambiguously establish a role for fungal morphogenesis as a virulence factor[126]. In addition, as virulence genes are co-regulated with cell morphogenesis, the ability to switch from yeast to hyphal growth in response to various environmental signals is considered to be inherent to *Candida* virulence. Both yeast and hyphal forms of fungi clearly have a wide range of attributes that contribute actively to fungal infectivity. Undoubtedly, fungal morphogenesis, through antigenic variability, phenotypic switching and dimorphic transition, implicates the existence of many recognition and effector mechanisms to oppose fungal infectivity at the different body sites.

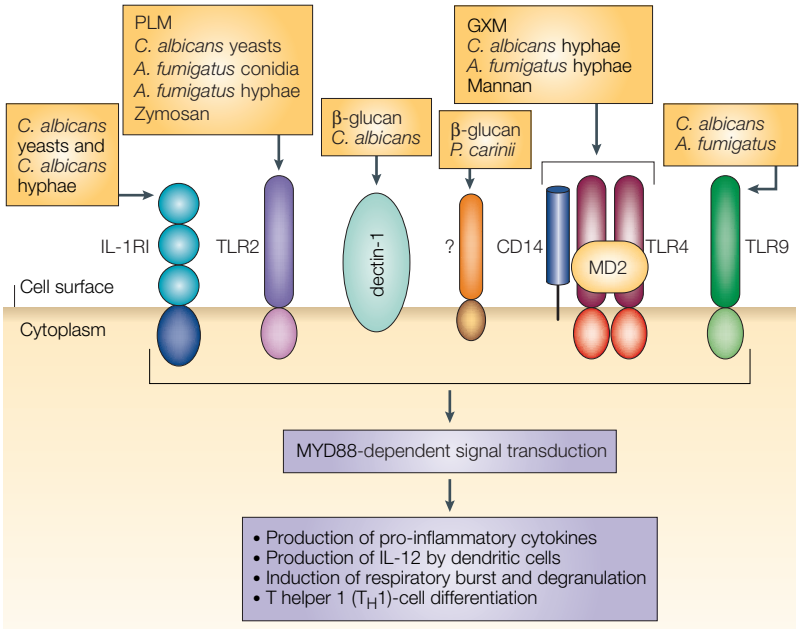


Figure 1 | **Role of TLRs as activators of innate and adaptive immunity to fungi.** The recognition of fungi and fungal pathogen-associated molecular patterns (PAMPs), mainly associated with fungal cell walls, leads to the activation of antifungal effector functions in phagocytes, such as respiratory burst and degranulation, and production of interleukin-12p70 (IL-12p70) by dendritic cells (see text). This leads to inflammatory and protective antifungal T helper 1 (T_H1)-cell responses. The canonical signalling pathway for mammalian Toll-like receptors (TLRs) and IL-1 receptor (IL-1R) after ligation of PAMPs involves interaction with the adaptor molecule MYD88 (myeloid differentiation primary response gene 88) located in the cytosol. The activation of the MYD88 adaptor culminates in the activation and nuclear translocation of nuclear factor-κB (NF-κB) and subsequent gene activation. Additional pathways, including the MYD88-independent pathway, are yet to be explored in response to fungi. GXM, glucoronoxylomannan from *Cryptococcus neoformans*; PLM, phospholipomannan from *Candida albicans*; *Aspergillus fumigatus*; *Pneumocystis carinii*.

CONIDIA
Externally borne asexual spores produced by filamentous fungi. Conidia of different shape and size easily disseminate into the environment.

DIMORPHISM
The ability of some fungi to cycle reversibly between yeast and hyphal forms.

OPSONINS
Substances, usually antibodies or complement components, that coat a particle such as a microorganism and enhance phagocytosis by phagocytic cells.

OXIDATIVE
The production of oxidizing agents, such as reactive oxygen and nitrogen intermediates, by effector phagocytes.

known to signal through the MYD88-dependent pathway[29]. It is of interest that *Aspergillus* hyphae, unlike CONIDIA, seem to be sensed by human monocytes through TLR4 and CD14 (REF. 30), which indicates that TLRs discriminate between distinct fungal morphotypes. However, as *Aspergillus* hyphae might also evade TLR recognition[31], this indicates that TLR recognition of only selected fungal morphotypes might contribute to the survival of fungi *in vivo*. TLR4 and CD14 also mediate the recognition of *Saccharomyces cerevisiae*- and *C. albicans*-derived mannan[32] and of glucoronoxylomannan[33] (a major component of the capsule of *Cryptococcus neoformans*). The finding that glucoronoxylomannan only partially activates TLR-dependent signal transduction pathways might account for its immunosuppressive and immunodysregulatory effects on the host. Although TNF and IL-1β production in response to *C. albicans* might also occur in a TLR4-independent manner, resistance to infection is decreased in TLR4-deficient mice, together with the release of chemokines[25]. Therefore, TLR2 and TLR4 are both involved in inducing host defences to the fungus, a finding that exemplifies the recruitment of different TLRs by one microbial species. Our own studies indicate that the MYD88-dependent pathway in DCs is required for adaptive T_H1-cell-mediated resistance to *C. albicans* and *A. fumigatus*[34]. The MYD88-dependent pathway is also essential for innate resistance to *C. albicans*, but not to *A. fumigatus*, which is in line with the unaltered handling of the fungus by MYD88-deficient macrophages[35]. Intriguingly, *Drosophila* Myd88, although required for antifungal defence, is unable to induce expression of the antifungal peptide drosomycin in the absence of other adaptors[36]. The contribution of individual TLRs to the immune response might vary depending on fungal species, fungal morphotypes and route of infection. For example, signalling by *C. albicans* essentially occurs through IL-1R (a finding that is consistent with the occurrence of IL-1 in infection[16]) and by *A. fumigatus* through TLR4, and TLR2 and TLR4 are both implicated in different ways in the control of disseminated or mucosal infections with *C. albicans*[34].

Individual TLRs and IL-1R also activate specialized antifungal effector functions of neutrophils that correlate with susceptibility to infection[34]. TLR expression by neutrophils exposed to *C. albicans* and *A. fumigatus* is induced in a morphotype-specific manner. Although they do not affect phagocytosis, TLRs affect specific antifungal programmes of neutrophils, such as the respiratory burst and degranulation[34]. As the quantity and specificity of delivery of toxic neutrophil products ultimately determine the relative efficiency of fungicidal activity versus inflammatory cytotoxicity to host cells, this indicates that TLRs might govern protection and immunopathology at the level of the innate immune response.

The emerging picture indicates an essential requirement for the IL-1R1–MYD88-dependent pathway in innate and T_H1-cell-mediated resistance to *C. albicans*, and the crucial, although not obligatory, involvement of the TLR4–MYD88 pathway in resistance to *A. fumigatus*. TLR signalling occurs in a morphotype-specific manner, although the simultaneous ligation of many TLRs, as well as TLR cooperativity *in vivo*, make it difficult to gauge the relative contributions of individual fungal morphotypes in TLR activation and functioning. Also, TLRs have different effects on the occurrence of innate and adaptive T_H1-cell immunity to each fungus, which is consistent with the ability of individual TLRs to activate specialized antifungal effector functions in neutrophils and DCs[34].

Antifungal effector activity. The antifungal effector functions of phagocytes include killing and growth inhibition of fungi, as well as pathways to oppose fungal infectivity, including effects on DIMORPHISM and phenotypic switching[16]. Although, in general, phagocytes have intrinsic antifungal activity, this activity can be increased by OPSONINS and T-cell-derived cytokines, which indicates that the innate and adaptive immune systems do not work independently, but are reciprocally regulated[14,16]. The optimal restriction of fungal growth occurs through a combination of OXIDATIVE and complementary non-oxidative mechanisms, the latter consisting of degranulation and intracellular or extracellular release of effector molecules, defensins and neutrophil cationic peptides, and iron sequestration[16]. Enzymes

CHRONIC GRANULOMATOUS DISEASE
A primary immunodeficiency that affects phagocytes. It is characterized by a greatly increased susceptibility to severe bacterial and fungal infections.

YEASTS
Unicellular oval or spherical cells, usually about 3 to 5 μm in diameter, that reproduce asexually by processes known as blastoconidia formation (budding) or fission.

such as the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and inducible nitric oxide synthase initiate the oxidative pathways known as respiratory burst. The respiratory burst produces toxic reactive oxygen intermediates (ROIs), the nature of which varies depending on the nature of pathogens and the type of phagocytic cell. ROIs damage fungi by producing protein modifications, nucleic-acid breaks and lipid peroxidations[37]. The production of ROIs is initiated by microbial products, such as lipopolysaccharide (LPS), and is potentiated by opsonins and cytokines[16]. In retaliation, fungi have evolved strategies to selectively inhibit the respiratory burst through the production of specific scavengers of oxidative killing by phagocytes, such as catalase, mannitol and melanin[38]. Patients with inherited X-linked CHRONIC GRANULOMATOUS DISEASE, resulting from a deficiency in oxidant formation due to mutations in any of the four genes that encode the subunits of NADPH oxidase[39], have increased susceptibility to aspergillosis, but could benefit from interferon-γ (IFN-γ) therapy[40]. Moreover, transplantation of bone-marrow cells transfected with the gene encoding NADPH oxidase restored fungicidal activity of mice with chronic granulomatous disease — a finding that opens up the possibility for gene therapy in fungal infections[41].

The fact that not only quantitative[42], but also qualitative[43] defects in neutrophils are important predisposing factors to certain disseminated fungal infections points to a functional versatility of neutrophils in fungal diseases. Their functions might well go beyond microbicidal activity, to include an immunoregulatory action on T_H cells. Accumulating evidence indicates that cells of the myeloid lineage are capable of positive and negative regulation of T-cell function[44]. Myeloid suppressor cells are responsible for the immunosuppression observed in pathologies as varied as tumour growth, overwhelming infections, graft-versus-host disease and pregnancy[44]. A population of neutrophils that suppress T_H1-cell activation is present in bone-marrow-transplanted mice with candidiasis[45], which indicates that myeloid suppressor cells might prevent functional immunoreconstitution in transplantation. The reciprocal influence between neutrophils and T cells further implies that immunity to fungi is a highly coordinate and unitary process.

Macrophages are a heterogeneous population of tissue-resident cells that express the machinery for antigen presentation; however, their main contribution to antifungal defence is phagocytosis and killing of fungi[37,46]. Not surprisingly, therefore, fungi have various mechanisms or putative virulence factors to evade phagocytosis, escape destruction and survive inside macrophages[38,47–51]. Macrophages serve as a protected environment in which the dimorphic fungi multiply and disseminate from the lungs to other organs. *Histoplasma capsulatum* is an example of a successful intracellular pathogen of mammalian macrophages[50].

Complement, collectins and antibodies promote binding (opsonization) and recognition of fungi by various receptors[16]. A member of the collectin family, pentraxin 3, is required for prompt handling of *Aspergillus* conidia by alveolar macrophages, such that its deficiency is linked to the susceptibility to infection of otherwise immunocompetent mice[18]. The specific biological activities of the complement system and antibodies that contribute to host resistance are multifaceted and interdependent[52,53]. For example, antibodies contribute to the activation of the complement system by fungi[52] and complement is essential for antibody-mediated protection[54]. Studies with *C. neoformans* have shown that the high levels of carbon dioxide in the lungs favour capsule formation, which impairs phagocytosis in the absence of anticapsular antibodies[55] that can alter the conformation of the capsule and so favour direct binding and phagocytosis of YEASTS[56,57]. However, antibodies have shown disparate biological effects in fungal infections — a finding that is consistent with the evidence that both protective and non-protective antibodies are induced during infection[53,58]. For this reason, interest in antibodies has recently seen a resurgence to identify those that positively modulate infection (BOX 3). Complement, antibodies and collectins not only fulfil the requirement of a first line of defence against fungi, but also have an impact on the inflammatory and adaptive immune responses, through several mechanisms, including regulation of cytokine secretion and co-stimulatory molecule expression by phagocytes[59,60].

Each receptor on phagocytes not only mediates distinct downstream intracellular events related to clearance of fungi, but it also participates in complex and disparate functions related to immunomodulation and activation of immunity, depending on the cell type. The receptors for different complement breakdown products (complement receptors, CRs), mannosyl–fucosyl glycoconjugate ligands (mannose

Box 3 | **Antibodies in immunity to fungi**

The main recognized functions of antibodies in fungal infections include: prevention of adherence, toxin neutralization, opsonization and antibody-dependent cellular cytotoxicity[53]. However, the absence of an association between deficiencies in antibodies and susceptibility to fungal infections and the presence of specific antibodies in patients with progressive fungal infections[53] have provided evidence against a protective role of antibodies in fungal infections. Recent advances in the field show that:

- **both protective and non-protective antibodies against fungi can be shown, the relative composition and proportion of which might vary greatly[53,58,127];**
- **the amount, specificity, isotype and idiotype of antibodies have marked effects on protective efficacy[53];**
- **antibodies specific for heat-shock protein 90 are associated with recovery from infections with *Candida albicans* and protection against disseminated disease in patients with AIDS, and they synergize with antifungal chemotherapy[128];**
- **antibodies specific for a mannan adhesin fraction passively transfer protection against candidiasis in mice[129];**
- **idiotype-specific antibody or single chains thereof have broad fungicidal activity and therapeutic efficacy in experimental infections[130,131].**

Research is now in progress to identify antibodies that are protective, the peptide mimetics that specifically elicit them and putative candidate vaccines that elicit protective immunity. Nevertheless, given the interdependency between humoral and cellular immunity in infections, a division of labour might be conceptually inconsistent with the experimental observations that an intact T-cell function is required for antibody-mediated protection and that antibodies might have an influence over the T_H1/T_H2-type cytokine balance and the induction of regulatory T cells.

receptors, MRs) and β-glucan (dectin-1) act as early warning systems and, not surprisingly, their ability to activate, in isolation, various effector functions is limited. With a few exceptions[61], internalization through constitutively competent MRs does not lead to effective clearance of fungi in the absence of opsonins. However, MRs expressed by DCs activate specific programmes that are relevant to the development of antifungal immune responses (see later). Ligation of CR3 (also known as CD11b/CD18) is one of the most efficient means of engulfing opsonized fungi, but it also has broad recognition capacity for diverse fungal ligands. The multiplicity of binding sites and the existence of different activation states enable CR3 to engage in disparate (positive and negative) effector activities against fungi. So, because signalling through CR3 might not lead to phagocyte activation without the concomitant ligation of receptors for the Fc portion of immunoglobulins (FcRs), this might contribute to intracellular fungal parasitism. It is of interest, therefore, that *H. capsulatum* uses this receptor to gain entry into macrophages, where it survives[62], and not into DCs[63], where it is rapidly degraded. Similarly, *Candida* enters through CR3 to survive inside DCs (discussed later). By contrast, ligation of FcRs is usually sufficient to trigger phagocytosis, a vigorous oxidative burst and the generation of pro-inflammatory signals. Ultimately, the recognition of antibody-opsonized particles is a high-level threat. It is of interest that FcR-mediated phagocytosis might rescue suppression of the respiratory burst, which indicates one possible mechanism by which T and B cells enhance the antifungal activity of macrophages and might explain the failure of macrophages from HIV-infected subjects to oppose fungal infectivity efficiently (reviewed in REF. 16).

The instructive role of innate immunity. The instructive role of the innate immune system in the adaptive immune responses to fungi occurs through fungal growth restriction — as $CD4^+$ T_H-cell differentiation *in vivo* is affected by antigen load — expression of co-stimulatory molecules by phagocytic cells, and chemokine and cytokine production (reviewed in REF. 64). The local release of these effector molecules regulates cell trafficking by various types of leukocyte, therefore initiating an inflammatory response, activating phagocytic cells to a microbicidal state and directing T_H-cell development. The inflammatory response to fungi might serve to limit infection, but might also contribute to pathogenicity, as documented by the occurrence of severe fungal infections in patients with immunoreconstitution disease[65]. These patients might experience intractable fungal infections despite recovery from neutropaenia and the occurrence of adaptive immune responses. Therefore, recovery from infection might not only depend on effector-cell function, but also on resolution of the inflammatory process.

Transforming growth factor-β (TGF-β) and IL-10 are potent immunosuppressive cytokines, with beneficial and detrimental effects on host responses to fungi. IL-10 is a double-edged sword in the fight against fungi. IL-10 is readily produced by neutrophils, macrophages, DCs and REGULATORY T (T_{REG}) CELLS, and it has a crucial role in determining susceptibility to fungal infections. IL-10 is produced in a morphotype-specific manner and acts by impairing the antifungal effector functions of phagocytes, the secretion of pro-inflammatory cytokines, such as TNF, IL-1β, IL-6 and IL-12, and protective cell-mediated immunity. The finding that fungal pneumonia can occur after TNF-ablation therapy underscores the pivotal role of pro-inflammatory cytokines in the control of infection[66,67]. However, later in the course of an infection, high-level production of IL-10 might be beneficial by contributing to resolution of the inflammatory response. Circumstantial evidence indicates that IL-10 produced by cells of the innate immune system is responsible for the prevention of excessive activation of innate effector functions, whereas IL-10 secreted by T_{Reg} cells is mainly responsible for the establishment of commensalism and, perhaps, fungal latency and persistence[68].

Virtually all fungi that infect humans induce the production of IL-12 by phagocytes and DCs[69]. Similar to IL-10, IL-12 is produced in a morphotype-dependent manner, through the use of different recognition receptors and TLRs[34]. For example, its production is inhibited by CR3 ligation on macrophages by *H. capsulatum*[70] and on DCs by *C. albicans* (see later). IL-12, together with IL-18, induces IFN-γ — a key cytokine in the innate control of fungal infections in mice and humans[69]. IFN-γ, produced by T and NK cells, stimulates migration, adherence, phagocytosis and oxidative killing of neutrophils and macrophages, and sustains T_H1-cell reactivity by its ability to maintain IL-12 responsiveness in $CD4^+$ T cells. IFN-γ restores resistance to fungi in patients with chronic granulomatous disease[40] and, as an adjunctive therapy, potentiates the efficacy of antifungal chemotherapy[71]. Deficient IFN-γ receptor-mediated signalling occurs in neonates and might predispose to fungal infections[72]. However, experimental evidence indicates that IFN-γ might not work in a T_H2 setting[12] — a finding that underscores the requirement for critical interpretation of the levels of IFN-γ production in clinical settings.

DCs as an interface between host and fungi

DCs acquire antigens in peripheral tissues and, as they mature, migrate to the T-cell areas of lymphoid organs, providing T cells with the appropriate signals. As DCs express several TLRs, they are the main connectors of the innate and adaptive immune systems. DCs are uniquely adept at decoding the fungus-associated information and translating it into qualitatively different adaptive T_H-cell immune responses[73]. TLRs and other PRRs determine the functional plasticity of DCs in response to fungi and contribute to the discriminative recognition of different fungal morphotypes. DCs (both human and mouse) are now known to recognize and internalize several fungi, including *A. fumigatus*, *C. albicans*, *C. neoformans*, *H. capsulatum* and *Malassezia furfur*[14,37,73]. *C. albicans* proved to be a useful pathogen model to dissect events that occur at the fungus–DC interface[74,75]. A unique feature of DCs is

REGULATORY T (T_{REG}) CELLS
T_{Reg} cells are $CD4^+CD25^+$ cells that regulate the balance between immunity and immunopathology.

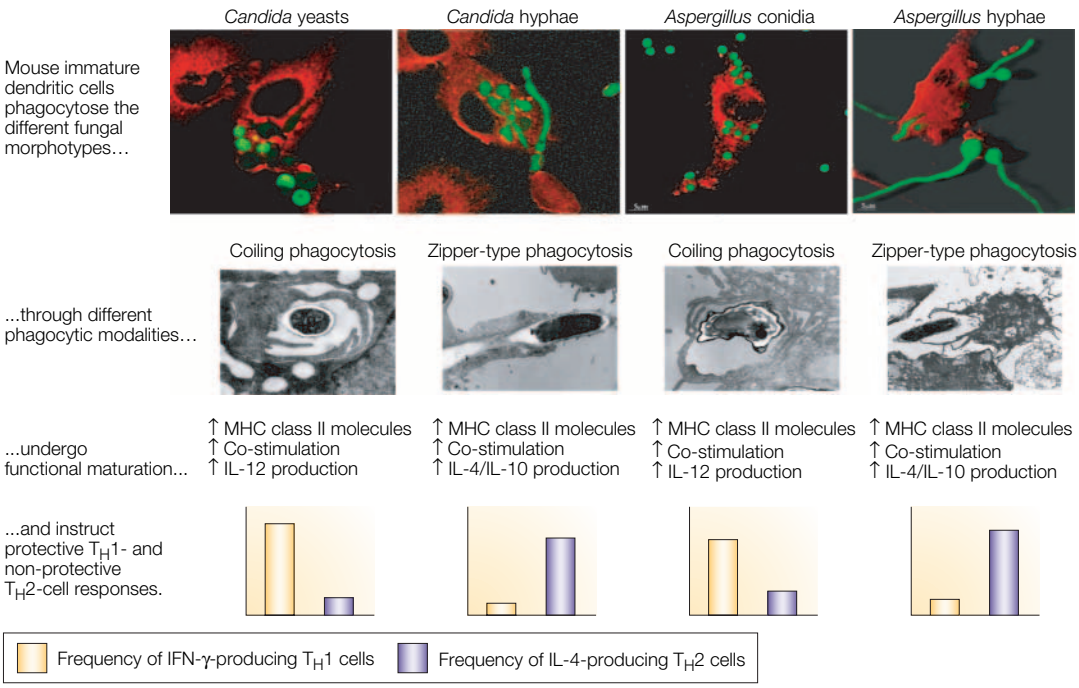


Figure 2 | **The interaction of dendritic cells with fungi: a host perspective of fungal virulence.** Dendritic cells (DCs) are unique phagocytic cells as they can phagocytose different fungal morphotypes of *Candida albicans* and *Aspergillus fumigatus*, as shown by the confocal microscopy (top panels). *Candida* images reproduced from REF. 74 with permission © The Rockefeller University Press (2003). *Aspergillus* images reproduced, with permission, from REF. 114 © The American Association of Immunologists, Inc. (2002). Transmission electronic microscopy indicates that the uptake of the different fungal elements occurs through different forms of phagocytosis. Internalization of yeasts and conidia occurs mainly by coiling phagocytosis, which is characterized by the presence of overlapping bilateral pseudopods, that leads to a pseudopodal stack before transforming into a phagosome wall. By contrast, entry of hyphae occurs by a more conventional, zipper-type phagocytosis, characterized by the presence of symmetrical pseudopods, which strictly follows the contour of the hyphae before fusion. As DCs are equipped with pattern-recognition receptors such as Toll-like receptors, they can decode the fungus-associated information and translate it into qualitatively different adaptive T helper (T_H)-cell immune responses. The ligation of distinct receptors by yeasts/condida and hyphae translates into downstream signalling events, ultimately regulating co-stimulation, cytokine production and the development of T_H and regulatory T cells — an event that is greatly influenced by fungal opsonins (see text). The functional plasticity of DCs at the pathogen–immune system interface might offer new clues to fungal virulence. IFN-γ, interferon-γ; IL, interleukin.

their ability to internalize different fungal morphotypes. For example, DCs internalize *Candida* yeasts, *Aspergillus* conidia and hyphae of both (FIG. 2). Phagocytosis occurs in distinct ways and involves different recognition receptors. Recognition and internalization of yeasts and conidia occurs mainly by COILING PHAGOCYTOSIS, through the ligation of MRs of different sugar specificity, DC-SIGN and, partly, CR3. By contrast, entry of hyphae occurs by a more conventional, ZIPPER-TYPE PHAGOCYTOSIS, and involves the cooperative action of FcγR and CR3 (REF. 68). Phagocytosis of either fungal form does not require TLR2, TLR4, TLR9 or MYD88 (REF. 34).

The ligation of distinct receptors by yeasts and hyphae translates into downstream signalling events, ultimately regulating cytokine production and co-stimulation — an event that is greatly influenced by fungal opsonins[68]. Entry through MRs results in the production of pro-inflammatory cytokines, including IL-12, upregulation of co-stimulatory molecules and MHC class II molecules, and activation of protective T_H1-cell responses. MRs are also required for the entry of *C. neoformans* into DCs and the activation of antifungal T_H1-cell responses[76,77]. These events are suppressed after entry of fungus through CR3. Co-ligation of CR3 with FcγR, as in the phagocytosis of hyphae or opsonized yeasts, results in the production of IL-4 and/or IL-10, upregulation of co-stimulatory molecules and MHC class II molecules, and activation of T_H2/T_{Reg} cells[68]. Signalling through the MYD88 pathway is required for the production of IL-12 by DCs in response to *Candida* yeasts and *Aspergillus* conidia with the implication of distinct TLRs (IL-1RI and TLR9 for *Candida* and TLR4 and TLR9 for *Aspergillus*); however, TLR2, but not MYD88, signalling is required for IL-10 production[34].

Opsonins greatly modify receptor usage on DCs by the different fungal morphotypes and qualitatively affect DC activation. Mannose-binding lectin (MBL) opsonization, for example, increases the uptake of yeasts through CR3 and prevents DC activation and the production of IL-12 (REF. 68). It is of interest that collectins seem to favour phagocytosis of fungus without inducing the production of cytokines — an activity that is compatible with a primitive mechanism of host defence and in line with their ability to down-regulate the inflammatory response to fungi[59]. These results might explain the increased susceptibility to

COILING PHAGOCYTOSIS
A mechanism for the uptake of eukaryotic microorganisms by phagocytic cells, in which unilateral pseudopods of the phagocytes wrap around microorganisms in multiple turns, giving rise to largely self-apposed pseudopodal surfaces.

ZIPPER-TYPE PHAGOCYTOSIS
A mechanism of phagocytosis of pathogens, which occurs by sequential interactions between receptors and ligands on the surfaces of the phagocytes and pathogens. Consequently, the engulfing pseudopods strictly follow the contour of the particle.

fungal infections of patients with defective MBL[78] or *MBL* gene polymorphisms[79]. Antibodies can also subvert the entry of yeasts and hyphae[68,80]. A notable and important feature of Peyer's patch DCs is the production of IL-10 in response to *Candida* — an event that occurs by signalling through CR3 and that requires the presence of opsonizing antibodies[80]. These IL-10-producing DCs activate $CD4^+CD25^+$ T_{Reg} cells that negatively affect antifungal T_H1-cell reactivity (see later). So, by subverting the morphotype-specific programme of activation of DCs, opsonins might qualitatively affect DC function and T_H-cell differentiation *in vivo*, ultimately impacting on fungal virulence. It is conceivable that tissue-dependent factors, opsonins and antibodies modulate receptor usage by DCs at different body sites, and might contribute to the functional plasticity of DCs at the pathogen–immune system interface.

In this scenario, the qualitative development of the T_H-cell response to a fungus might not mainly depend on the nature of the fungal form being phagocytosed and presented. Instead, the nature of the cell response is strongly affected by the type of cell signalling that is initiated by the ligand–receptor interaction in DCs. For *Candida*, this model would predict that dimorphism *per se* can no longer be considered as the single most important factor in determining commensalism versus infection, nor can specific forms of the fungus be regarded as absolutely indicative of saprophytism or infection at a given site. The selective use of receptor-mediated entry into DCs could explain the full range of host immune relationships with the fungus, including saprophytism and infection. Usage of CR3, and the consequent attenuation of IL-12 production, might favour commensalism of the fungus at human mucosal surfaces, including gut and vagina, where immune tolerance is desirable to the host. Averting cellular activation through interaction with CR3 might be an important evasive strategy for fungi. Finally, as both fungal morphotypes, but particularly hyphae, activate gut DCs for the local induction of T_{Reg}-cell responses[68], and because the morphogenesis of *C. albicans* is activated *in vivo* by a wide range of signals[81], it seems that the discriminative response towards T_{Reg}-cell function represents a successful strategy of adaptation to the mammalian host. It could indeed allow for fungal persistence in the absence of the pathological consequences of exaggerated immunity and possible autoimmunity. Therefore, in addition to the induction of phase-specific products that enhance fungal survival in the host, transition to the hyphal phase of the fungus could induce immunoregulatory events that will benefit the host[82].

Adaptive immunity

Serological and skin reactivity assays indicate that fungal infections are common, but clinical disease is rare, consistent with the development of acquired immunity[83]. For many fungal pathogens, the effective tissue response to invasion is granulomatous inflammation — a hallmark of cell-mediated immunity. For dimorphic fungi, the initial exposure is either asymptomatic or results in mild infection that confers protective immunity. For *C. neoformans*, the high prevalence of antibodies specific for cryptococcal antigens in normal individuals indicates that primary infection is followed by fungal growth restriction and lifelong immunity. Underlying acquired immunity to *C. albicans*, such as the presence of a positive DELAYED-TYPE HYPERSENSITIVITY (DTH) response, is demonstrable in adult immunocompetent individuals, and is presumed to prevent progression from mucosal colonization to symptomatic infection. Lymphocytes from healthy individuals show proliferative responses after stimulation with fungal antigens and produce several different cytokines[84–86].

There is marked plasticity in the T-cell response to fungi. The heterogeneity of the $CD4^+$ and $CD8^+$ T-cell repertoire might account for the multiplicity and redundancy of effector mechanisms through which T cells participate in the control of fungal infections. These include direct antifungal activity[87], release of antimicrobial peptides from $CD8^+$ T cells[88], lysis of fungus-containing phagocytes[89], and effector functions resulting from dynamic interactions with T cells that express selected members of the Vβ families of the T-cell receptor[90]. This functional plasticity indicates the potential of vaccines in conditions of immunodeficiency, as highlighted by the ability of $CD8^+$ T cells to compensate for $CD4^+$ T-cell deficiency in experimental models of vaccine-induced resistance to endemic fungi[91,92]. The flexible programme of T cells leads to the production of many mediators, including cytokines. Due to their action on circulating leukocytes, the cytokines produced by fungus-specific T cells are instrumental in mobilizing and activating antifungal effectors, so providing prompt and effective control of infectivity after the fungus has established itself in tissues or spread to internal organs. Therefore, host resistance to fungi seems to depend on the induction of cellular immunity, mediated by T cells, cytokines and effector phagocytes (FIG. 3).

The clinical circumstances in which fungal infections occur are associated with impaired cell-mediated immunity. AIDS and severe haematological malignancies are examples of acquired defects in T-cell function that predispose to severe fungal infections. Furthermore, the occurrence of severe disseminated infections by filamentous fungi in non-granulocytopaenic patients[2–4], as well as with the onset of graft-versus-host disease in bone-marrow-transplant recipients[93], provides compelling evidence of the pathogenic role of T-cell dysreactivity in infection. In endemic mycosis, the severity of the disease correlates with the degree of impairment of cell-mediated immunity, and is associated with increased levels of antibodies[94]. Generation of a dominant T_H1-cell response mediated by IL-12 is required for the expression of protective immunity to fungi. Experimental data have shown the deleterious effects of IL-12 or IFN-γ ablation on the course and outcome of fungal infections[69]. Through production of the signature cytokine IFN-γ and providing help for opsonizing antibodies, the activation of T_H1 cells is instrumental in the optimal activation of phagocytes at sites of infection. Therefore, the failure to

DELAYED-TYPE HYPERSENSITIVITY (DTH). A T-cell-mediated immune response characterized by monocyte/macrophage infiltration and activation. DTH skin tests have classically been used for the diagnosis of infection with intracellular pathogens such as *Mycobacterium tuberculosis*, and as a measure of the vigour of the cellular immune system. Classical DTH responses to intracellular pathogens are thought to depend on $CD4^+$ T cells that produce a T helper 1-type profile of cytokines (interferon-γ and tumour-necrosis factor).

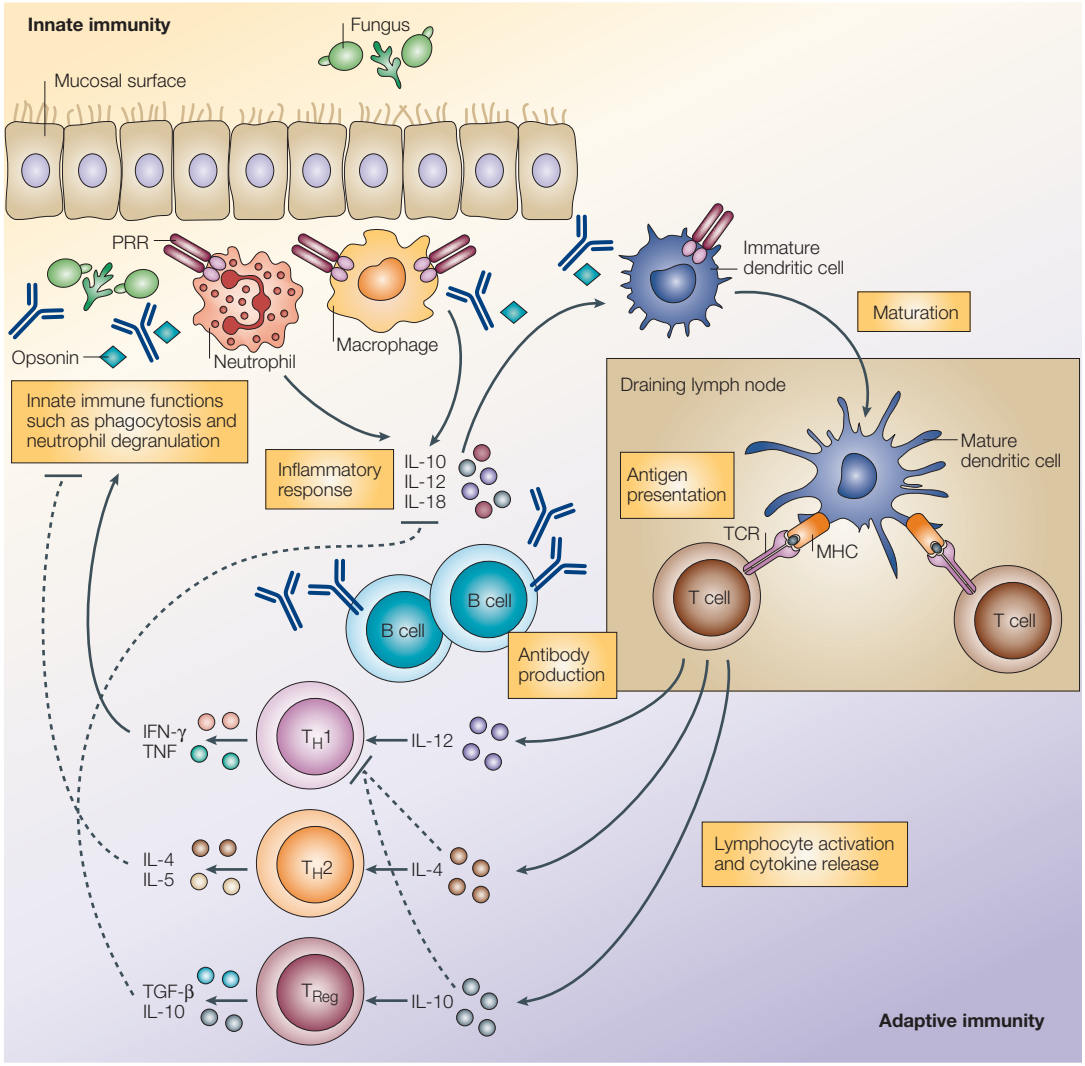


Figure 3 | **Balancing protection and immunopathology in fungal infections: a cooperative effort of the innate and adaptive immune systems.** Most fungi are detected and destroyed within hours by innate defence mechanisms mediated by phagocytes and opsonins through the involvement of distinct pattern-recognition receptors (PRRs). These mechanisms act immediately and are followed some hours later by an early induced inflammatory response, which must be activated by infection but does not generate lasting protective immunity. These early phases help to keep infection under control. In vertebrates, however, if the infectious organism can breach these early lines of defence, an adaptive immune response will ensue, with the generation of antigen-specific T helper (T_H) effector cells, regulatory T (T_{Reg}) cells and B cells that specifically target the pathogen and induce memory cells that prevent subsequent infection with the same microorganism. Dendritic cells sample fungi at the site of colonization/infection, transport them to the draining lymph nodes and activate disparate T_H and T_{Reg} cells in a morphotype- and tissue-dependent manner. As the different T_H-cell subsets release a distinct panel of cytokines, capable of delivering activating and inhibitory feedback signals to effector phagocytes, the activation of the appropriate T_H-cell subset is instrumental in the generation of a successful immune response to fungi. Counter-regulatory T_{Reg} cells might serve to dampen the excessive inflammatory reactions and contribute to the development of memory antifungal immunity. Solid and broken lines refer to positive and negative signals, respectively. IFN-γ, interferon-γ; IL, interleukin; TCR, T-cell receptor; TGF-β, transforming growth factor-β; TNF, tumour-necrosis factor.

deliver activating signals to effector phagocytes might predispose patients to overwhelming infections, limit the therapeutic efficacy of antifungals and antibodies, and favour persistency and/or commensalism. Immunological studies in patients with polarized forms of paracoccidioidomycosis show an association between T_H1-biased reactivity and asymptomatic or mild forms of the infection, in contrast to the correlation between T_H2-cell responses and severe disease[95]. Not surprisingly, therefore, patients with disseminated infection have defective production of IFN-γ and DTH anergy, associated with increased levels of type 2 cytokines (IL-4 and IL-5), IgE, IgG4 and IgA, and eosinophilia, which is a marker of poor prognosis in endemic mycoses[96]. In patients with a defective IL-12/IFN-γ pathway, such as those with hyperimmunoglobulinaemia E syndrome, fungal infections and allergy are both observed[97].

IL-4 is an important determinant of susceptibility and resistance in most fungal infections[11]. Ablation of IL-4 enhances immunity to fungi in experimental

models of infection[11]. IL-4 can both deactivate and activate phagocytes and DCs for certain specialized functions; for example, it can inhibit the antifungal effector activities of phagocytes, yet can promote the production of IL-12 by DCs[12]. So, the most important mechanism underlying the inhibitory activity of IL-4 in infections is its ability to act as the most potent proximal signal for commitment to T_H2-cell reactivity, which dampens protective T_H1-cell responses and favours fungal allergy. In atopic individuals, the suppressed DTH response to fungi is associated with increased levels of antifungal IgE, IgA and IgG[98,99]. However, susceptibility to fungal infections might not always be associated with marked production of IL-4. For example, although an association between chronic disseminated candidiasis and genetic variants of IL-4 has been described recently[100], levels of IL-4 or IL-5 are not always increased in patients with chronic mucocutaneous candidiasis (CMC), despite defective production of type 1 cytokines[101].

Several clinical observations indicate an inverse relationship between IFN-γ and IL-10 production in patients with fungal infections. High levels of IL-10, negatively affecting IFN-γ production, are detected in chronic candidal diseases[102], in the severe form of endemic mycoses[103] and in neutropaenic patients with aspergillosis[104]. Fungal polysaccharides are known to negatively modulate CMC through the production of IL-10, which indicates that IL-10 production might be a consequence of infection[11]. However, tolerance to fungi can also be achieved through the induction of T_{Reg} cells that can finely tune antifungal T_H-cell reactivity. Therefore, suppressor T cells have recently regained their reputation as key controllers of antifungal immunity[83]. Naturally occurring downregulatory mechanisms that occur in the respiratory mucosa might account for the lack of pathology in *P. carinii*-infected mice[105]. Administration of $CD4^+CD25^+$ T_{Reg} cells prevents the inflammatory pathology that is associated with pathogen clearance. In mice with candidiasis, $CD4^+CD25^+$ T_{Reg} cells, producing IL-10 and TGF-β, prevent complete elimination of the fungus from the gastrointestinal tract; fungal persistence allows the development of memory immunity[68].

It has long been presumed that the ability of *C. albicans* to persist in host tissues mainly involves the immunosuppressive property of cell-wall glycoproteins. Mannan and its oligosaccharide fragments could be potent inhibitors of cell-mediated immunity and seem to reproduce the immune deficit of patients with CMC[106]. CMC, although encompassing various clinical entities, has been associated with autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy — a condition in which the mutated gene has been shown to be involved in the ontogeny of $CD25^+$ T_{Reg} cells[107]. In CMC, the defective production of type 1 cytokines does not occur concomitantly with the increase in type 2 cytokine production (that is, IL-4 or IL-5) but, more often, with IL-10 (REF. 101). This finding has led to the speculation that an inherent alteration in receptor-mediated signalling in response to fungal polysaccharide might predispose patients with CMC to a dysfunctional induction of T_{Reg}-cell activity, negatively affecting T_H1-cell-dependent clearance of the fungus, without implicating the activation of T_H2 cells. Because both the recovery of fungus from the gastrointestinal tract and the detection of underlying T_H1-cell reactivity, such as DTH and lymphoproliferation, can fluctuate in healthy subjects, it is tempting to speculate that T_{Reg} cells mediate tolerance to the fungus at the site of colonization. The ligation of distinct TLRs on DCs and the ensuing production of IL-6 are crucial events that mediate the inhibition of T_{Reg}-cell function[20]. This is in agreement with the failure of IL-6-deficient mice to activate antifungal T_H1-cell responses concomitantly with increased IL-10 production[11]. It is also interesting that pro-inflammatory cytokines, but not IL-6, are produced by oral and/or vaginal epithelial cells in response to the fungus — a finding that might explain the downregulation of IFN-γ production in some patients with recurrent vaginal candidiasis[108].

Therapeutic prospects

One strategy to prevent antifungal drug resistance is to improve the immune functions of immunocompromised hosts. The therapeutic efficacy of antifungals is limited without the help of host immune reactivity[109]. Various cytokines, including chemokines and growth factors, have proved to be beneficial in experimental and human fungal infections[11]. The T_H1/T_H2-cell balance itself can be the target of immunotherapy. The inhibition of T_H2-type cytokines, or the addition of T_H1-type cytokines, can increase the efficacy of antifungals, such as polyenes and azoles, in experimental mycoses. The discovery of TLRs, which are now targets of antifungal drugs[110], DCs and their functional plasticity[68] and new roles for antibodies[57] have been major breakthroughs in the field of fungal immunology, which might offer new grounds for a better comprehension of the cells and immune pathways that are amenable to manipulation in patients with or at risk of fungal infections. Further understanding of the cooperation of various innate immune receptors in fungal recognition potentially provides a basis for new therapeutic strategies for immunomodulation. Notwithstanding the redundancy and overlapping repertoire of antifungal effector mechanisms, the deliberate targeting of cells and pathways of antifungal cell-mediated immunity might be a useful strategy in developing fungal vaccines capable of both sterilizing immunity and protection against fungal reactivation[111]. A great deal of attention is being focused on antigens that activate DCs to trigger these responses[112]. The ultimate challenge will be to design fungal vaccines that can induce optimal immune responses by targeting specific receptors on DCs *in vivo*. This will require, however, further studies aimed at elucidating the convergence and divergence of pathways of immune protection elicited in infections or after vaccination.

Studies *in vivo* confirm that DCs sample fungi at sites of infection, transport them to the draining lymph nodes and initiate disparate T_H-cell responses to the different fungal morphotypes[80,113–115]. Furthermore, adoptive transfer of DCs transfected with fungal RNA restores protective antifungal immunity in a mouse model of allogeneic bone-marrow transplantation[116]. These results, together with the finding that fungus-pulsed DCs can reverse the T-cell anergy of patients with fungal diseases[117,118], indicate the use of DCs for fungal vaccines.

More recently, research on fungi has entered the era of genomics[119]. Sequencing of the genomes of most fungal pathogens is almost complete. The emerging genomic sequence from many fungal species has already allowed the application of genomic technologies, such as DNA microarray analysis and signature-tagged mutagenesis, to the study of fungal pathogenesis, virulence and antifungal discovery[120,121]. It will be exciting to witness the revolution built on the combination of genomics with more traditional approaches in our understanding of fungi and fungal diseases.

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Competing interests statement
The author declares that she has no competing financial interests.

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